Studies on Insect Pigment Synthesis Pathways and their Application to Transgenic Markers

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Ommochromes and melanins are major pigments involved in reddish and blackish coloration in insects. However, molecular mechanisms of the synthesis of these pigments have not been fully understood. Here we report our findings on pigmentation mutants of silkworm *Bombyx mori* and their application to visible molecular markers.

We succeeded identifying the gene responsible for the *B. mori* egg and eye color mutant, *red egg* (*re*) by positional cloning, mutant analysis, and RNAi experiments [1]. The eggs of *re* mutant are red instead of normal dark purple, and have been suggested to have a defect in the biosynthesis of the final ommochrome pigments. The gene responsible for the *re* mutant was a novel major facilitator superfamily transporter. Interestingly, the *re* gene homolog were found in most insect genomes sequenced at present, but not in *Drosophila* genomes, which seemed to correlate with the previous studies that have demonstrated that eye ommochrome composition is different from other insects in several Dipterans. We also found that *B. mori cardinal* gene encoding a haem peroxidase was responsible for the *pink-eyed white egg* mutation, *pe* [2]. Eggs and eyes of *pe* mutant have a very small amount of pigments, due to defect in converting the precursor 3-hydroxykynurenine into various ommochrome pigments, suggesting the function of *cardinal* as a key synthesis enzyme for multiple ommochrome pigments.

Utilizing pigment synthesis pathway genes, we established two novel marker systems for insect transgenesis. By introducing the functional *Bm-re* gene into the *re* mutant strain, we succeeded in rescuing the egg coloration from mutant red to normal purple. This egg coloration marker allows transgenic screening without fluorescent microscopy, and also the egg coloration phenotype can be detected from an early stage compared with the conventional eye fluorescent markers. We also succeeded in developing a dominant visible transgenic marker, using *B. mori arylalkylamine-N-acetyl transferase* (*Bm-aaNAT*) gene [3]. This marker changed the color of newly hatched first-instar silkworm larvae from normal black to a distinctive light brown color. In addition, ectopic *Bm-aaNAT* expression also lightened coloration in ladybird beetle *Harmonia axyridis* and fruit fly *D. melanogaster*, highlighting its potential usefulness for transgenesis in diverse insect taxa.

References

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